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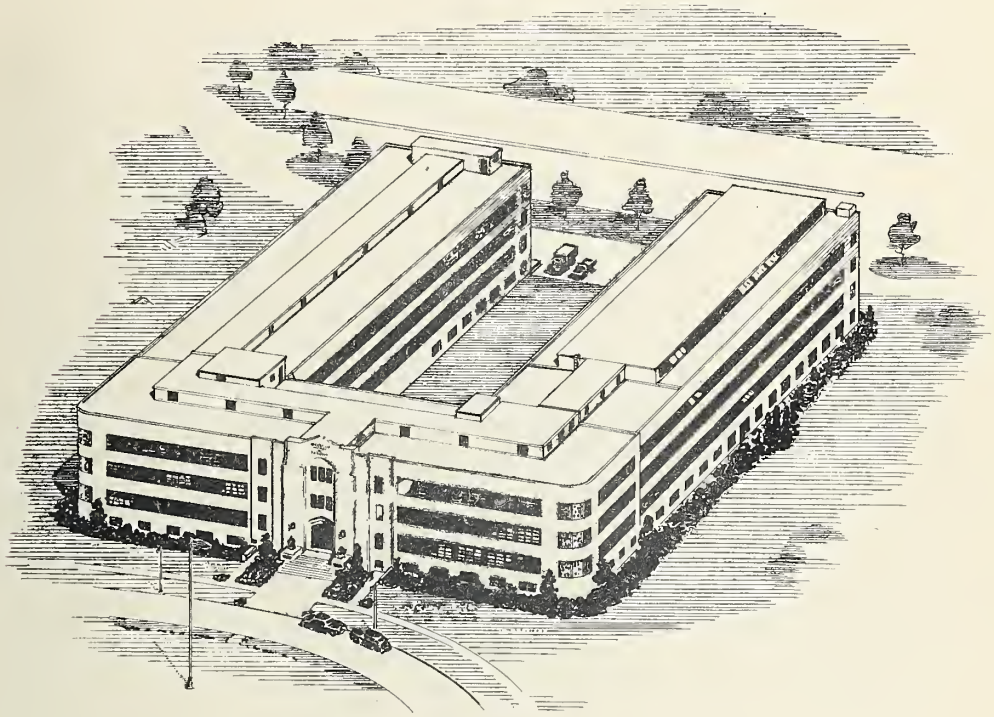
CHEMISTRY, PHARMACOLOGY, AND CLINICAL APPLICATION OF RUTIN

J. F. Couch, C. F. Krewson and J. Naghski



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By J. F. Couch, C. F. Krewson and J. Naghski

CONTENTS

INTRODUCTION

In 1936, Szent-Györgyi obtained evidence of a factor in citrus fruits and Hungarian red peppers distinct from ascorbic acid; he designated it vitamin P (7, 114) because of its action in remedying increased capillary permeability and fragility. Extensive chemical investigations by European chemists failed to isolate and identify the active material but did indicate its close relationship to the group of flavanone and flavone derivatives. To this day the chemical nature of vitamin P remains unknown.

The use of rutin to restore increased capillary fragility and permeability in man to normal was established in 1943 (61) by Griffith, Lindauer and Couch in a report to the Medical Society of the State of Pennsylvania. This was followed by publication the following year of experiences with 14 patients (59). Since that time, numerous papers have appeared in various medical, pharmacological and chemical journals concerning the properties of rutin and its use in hemorrhagic and other conditions. The substance is being manufactured by pharmaceutical companies and is available in tablets at most drug stores. Although several other compounds have been stated to have vitamin P properties, few of these are available in quantity and they do not seem to offer special advantages over rutin. The situation is well summed up by Dr. Kuhnau of the Physiological-Chemical Institute of Hamburg University: (155) "... . . . the physician has in rutin a vitamin P active substance at his disposal which is easily accessible, stable against air, heat, weak acids and alkalies, nontoxic, of exact dosage and highly active by mouth."

Rutin has the advantage of being a definite and uniform chemical compound of known structure that can be administered in precise dosage. A selected bibliography on rutin and related compounds has been prepared and may be obtained on application to this laboratory (24).

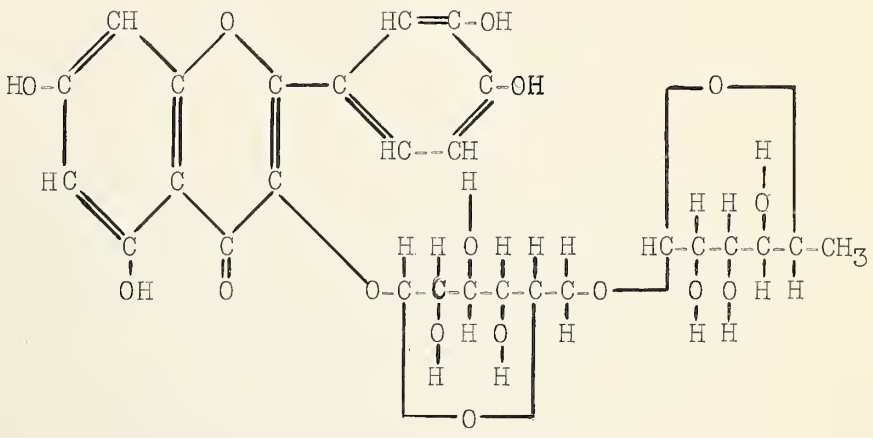
CHEMISTRY OF RUTIN

Rutin has been known for more than a century, having been discovered in 1842 by a Nuremburg apothecary, August Weiss (131), who obtained it from garden rue, *Ruta graveolens*, whence its name. It was soon discovered in other plants and is now known to be one of the most widely distributed glycosides in the plant kingdom. Until recent years there was no use known for this interesting compound. Plants containing it were formerly used in dyeing mordanted textile fibers, but that application ceased with the advent of the synthetic dyestuffs. In 1942 rutin was still a laboratory curiosity.

Rutin, $C_{27}H_{30}O_{16}.3H_2O$, is a flavonol glycoside, which can be prepared in a highly purified state. It is a yellow, tasteless, nontoxic powder consisting of masses of microscopic needle-shaped crystals soluble in boiling water (5 to 6 g. per l.) but only slightly in cold water (approximately 0.1 g. per l.) and easily purified by crystallization from this solvent. It is more soluble in alcohols (methanol, ethanol, isopropanol, etc.), ketones (acetone, methyl ethyl ketone), pyridine and alkaline solutions. It is insoluble in chloroform, ether, benzene and petroleum solvents.

Rutin crystallizes from water with three molecules of water of crystallization, which can be removed with varying degrees of ease. The third molecule requires vacuum, high temperature and an efficient desiccant. Rutin crystallizes from methanol, ethanol and acetone also with solvent of crystallization, which is rapidly given off in the air.

The microcrystallographic properties of rutin, quercitrin and quercetin have been described by Keenan (75). On hydrolysis with dilute acids, rutin yields a molecule each of the flavonol quercetin and the sugars glucose and rhamnose. The formula is therefore $C_{27}H_{30}O_{16}$, and the molecular configuration has been determined to be (8)



Rutin

CHARACTERIZATION

Rutin does not melt at a definite temperature. When heated, the crystals become plastic between 185 and 192° C. and appear to decompose above 214°. The melting point is, therefore, not a good criterion of purity.

Identification of the hydrolytic products is a more reliable means of characterization. The quercetin has a melting point of 312-314° C., and its pentaacetyl derivative melts at 194-196°. The two sugars can be characterized as the phenylosazones.

A valuable method of characterization and analysis is the determination of the absorption spectrum in the ultraviolet. Rutin shows maxima at 257.5 and 362.5 mμ, with specific extinction coefficients of 38.0 and 32.6, respectively. However, in the use of the absorption spectrum, it is necessary to keep in mind that quercetin, quercitrin and other quercetin derivatives have spectra similar to that of rutin.

Gage and Wender (146, 147, 166) have reported on the paper chromatography of rutin and several other flavonoids. They find it possible by this technique to separate the various compounds readily. A very extensive consideration of the chromatographic relationships of the flavonols and their glycosides and congeners has been published by Bate-Smith and Westall (141).

Various color tests have been used for detection of rutin; however, most flavones, flavanones, and flavonols give similar tests. Rutin dissolves in alkaline solutions with the formation of an intense yellow color. With alcoholic or aqueous ferric chloride rutin gives an intense green color. With acid and magnesium in alcoholic solution rutin gives a red color.

Rutin forms colored complexes with many heavy metals. This property is made use of for its analytical determination (103, 104). The ability to chelate with metals such as iron and copper, a property common to the flavonols, may also be responsible in part for its physiological activity (18, 20).

Rutin forms a fluorescent borocitric complex. Techniques based on this have been advocated for quantitative determination (53, 132), but are not entirely satisfactory.

OCCURRENCE

Rutin is widespread in nature, having been reported from approximately 40 species of plants. Among these are the buckwheats (*Fagopyreum esculentum* and *tataricum*), Chinese scholor tree (*Sophora japonica*), yellow pansy (*Viola tricolor*), elder (*Sambucus canadensis*), forsythia (*Forsythia suspensa* and *fortunei*), tobacco (*Nicotiana tabacum*), and asparagus (*Asparagus officinalis*).

Rutin was originally produced in this laboratory from flue-cured tobacco, which usually contains 0.3-0.5 percent rutin (25). However, the low yields from an expensive starting material made it essential to find a more economical source. Investigation of many plants revealed that buckwheat was a promising domestic raw material (30), since it contained 3 to 6 percent on a dry-weight basis. Of the several species commonly grown for grain, the Tartary (*F. tataricum*) is superior to the Japanese and Silver Hull (*F. esculentum*) as a source of rutin (31). The major portion of the rutin occurs in the leaves and blossoms and reaches a maximum in 35 to 45 days. The rutin can be extracted either from the fresh plant (27, 28) or from a meal prepared by artificial drying of the plant (42, 43, 79, 100).

DETERMINATION[1]

In plants: Dried plants are defatted with ether and extracted with alcohol: fresh plant is extracted with alcohol directly. The alcohol extract is evaporated, and the residue is boiled with water and filtered. The rutin crystallizing from the filtrate is filtered off in a Gooch crucible and dried at 110° C. Accuracy is increased by defatting the crude rutin with benzene and removing materials insoluble in absolute ethanol.

[1] More detailed discussions may be found in AIC-159 (102), AIC-236 (98) and W. B. Davis (36). These are summarized here.

In rutin preparations or pharmaceuticals: About 20 mg. of rutin is dissolved in 5 ml. of absolute ethanol, and adjusted to a volume of 100 ml. with 95 percent ethanol. When excipients are present, as in tablets, the rutin must be extracted. Ten tablets are weighed and powdered, and a sample containing about 20 mg. of rutin is weighed into a centrifuge tube. This is dispersed in 0.5 ml. of water and extracted with 25 ml. of 95 percent ethanol. The residue is washed twice by repeating the extraction procedure. The combined extract and washings are made up to 100 ml. with 95 percent ethanol. After further 16-fold dilution with 95 percent ethanol, 1 ml. of 0.02 N acetic acid is added, and the spectral densities are determined at wave lengths 362.5 and 375.0 mμ. If the $D_{375.0}/D_{362.5}$ ratio is 0.875 ± 0.004, the preparation is free of quercetin. If the density ratio is higher than 0.879, an appreciable quantity of quercetin is present. The rutin and quercetin are calculated by using extinction coefficients at these wave lengths (102). Swann (130) suggested that the determination of spectral densities at 347.0 and 375.0 mμ gives a more accurate determination of the quercetin.

In urine: To 2 ml. of urine are added 3 ml. of 0.1 M $AlCl_3$ and 0.5 ml. of 1.3 NH_4OH. The mixture is diluted to 10 ml. with water, centrifuged, and the supernatant is decanted. The gel is washed twice with dilute NH_4OH, drained, and dissolved by treating overnight with 0.1 ml. of glacial acetic acid. The solution is diluted with 10 ml. of 1 N potassium acetate, transferred to a 50-ml. volumetric flask and made to volume with water. After 30 minutes and not more than 2 hours, the optical density of the solution is determined at 413-416 mμ in a 5-cm. cell against a reagent blank carried through the same procedure (103).

PREPARATION AND REFINING

An early publication (25) described the preparation of rutin from tobacco by percolation with ethyl alcohol. The first rutin from buckwheat was prepared by cold solvent extraction of green plant, as described in later publications (27, 29). Proper drying conditions for the preparation of a buckwheat leaf meal were developed (42), and the meal was then extracted with either cold solvent or hot water as described in the same publication. A later circular described the preparation and refining of rutin from both green plant and meal by use of hot solvents (28). A revision (43) described improvements in drying conditions and hot water extraction technique. A final comprehensive paper on production of rutin from buckwheat (79) described the optimum preparative and refining techniques, presenting many of the data upon which recommendations to the rutin manufacturers were made.

The most efficient method for preparing and refining rutin from buckwheat may be described briefly as follows.

Either fresh green buckwheat or buckwheat leaf meal is placed in a suitable extractor and covered with isopropyl alcohol (80-85 percent strength by volume for green plant; 70-85 percent strength for buckwheat meal). The mixture is heated to boiling and, after a short interval (10 minutes), is pumped to an evaporator. Extraction of rutin is complete, no agitation is

necessary, and the marc need only be washed several times with hot solvent to remove rutin dissolved in entrained solvent. In an evaporator, the extract and washings are concentrated until the solvent is removed (about one-tenth original volume). If the process is operated under reduced pressure sufficient boiling water should be added before or during evaporation to about double the water already contributed by the dilute solvent used in the extraction. This is to prevent precipitation of rutin from the supersaturated concentrate. Under atmospheric pressure, the temperature during the evaporation is high enough to prevent rutin precipitation, and boiling water (2-3 volumes) is added after evaporation of the solvent..

The boiling concentrate is then strained through a glass-wool filter into a holding tank to remove the bulk of fats (26). Final traces of fat are removed by filtering through heavy paper filter pads, and the concentrate is then cooled to effect rapid crystallization of crude rutin.

After 1-2 hours, the cold crude rutin is filtered off on canvas or on a hard filter (preferably rayon paper) and either dried for storage or immediately refined. Refining of crude rutin is accomplished by dissolving in boiling water (about 18-20 gallons to the pound of dried crude rutin), filtering to remove finely divided insoluble material, and then treating the boiling rutin solution with silica gel (about 1/2 pound silica gel per pound of dried crude rutin) to remove so-called "red pigment" impurity[2]. The silica gel is filtered off, and the rutin rapidly cooled to effect crystallization. The product is dried to constant weight at 110° C. The process is represented diagrammatically in Figures 1 and 2. Figure 1 represents the extraction of crude rutin from buckwheat. Figure 2 illustrates the process of refining the crude rutin to a medicinal grade product.

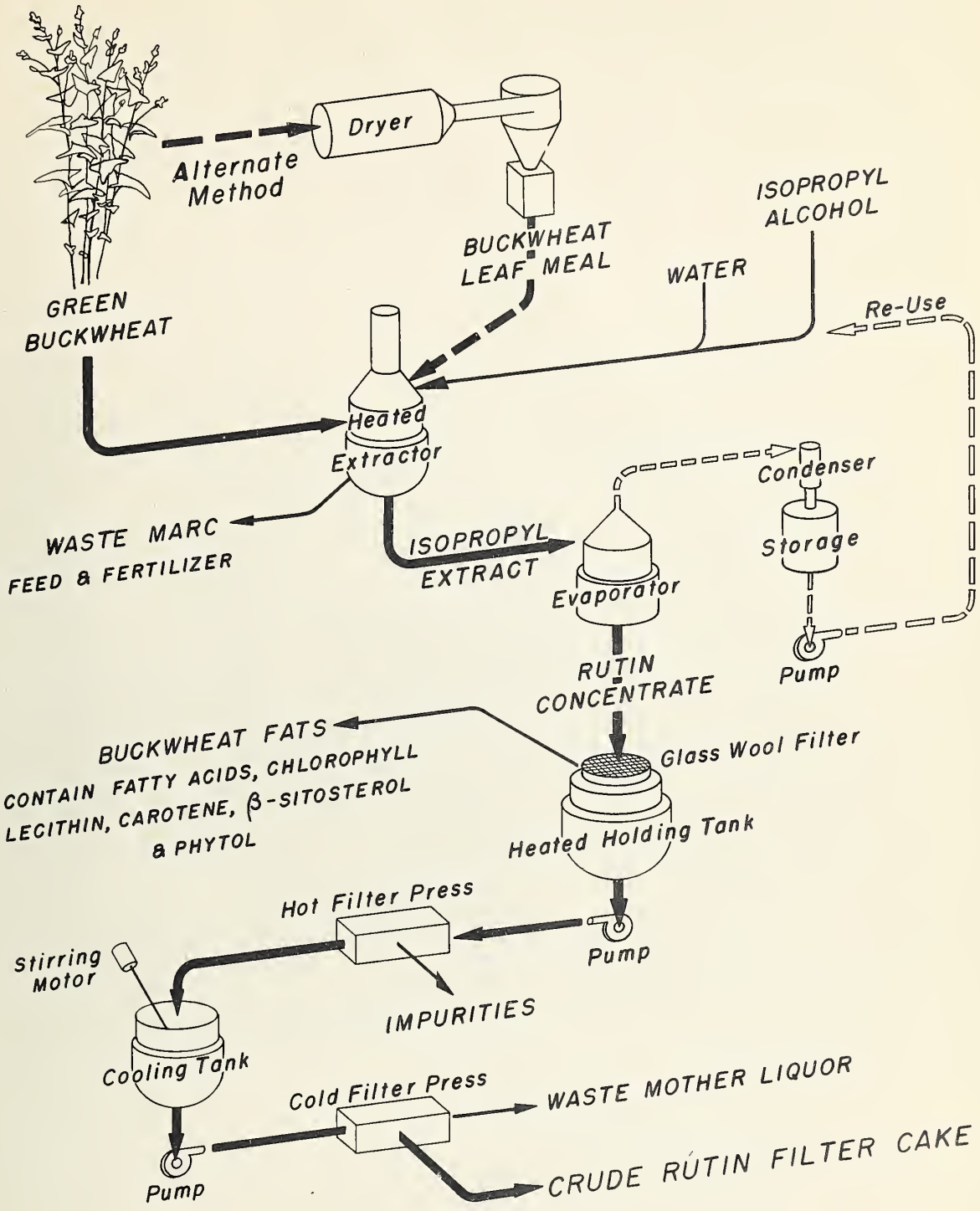
If the rutin does not meet specifications as outlined, it usually contains alcohol-insoluble impurities and may be recrystallized from isopropyl alcohol for their removal. The powdered, thoroughly dried rutin is dissolved in boiling isopropyl alcohol (1.75 gallons of 98-99 percent solvent to each pound of dry rutin), cooled to room temperature, and filtered through heavy asbestos pads. The rutin may be obtained by adding the alcohol filtrate to 10 volumes of water, or by adding the filtrate to boiling water so that the solvent may be distilled off and recovered for reuse in extraction.

PHARMACOLOGY

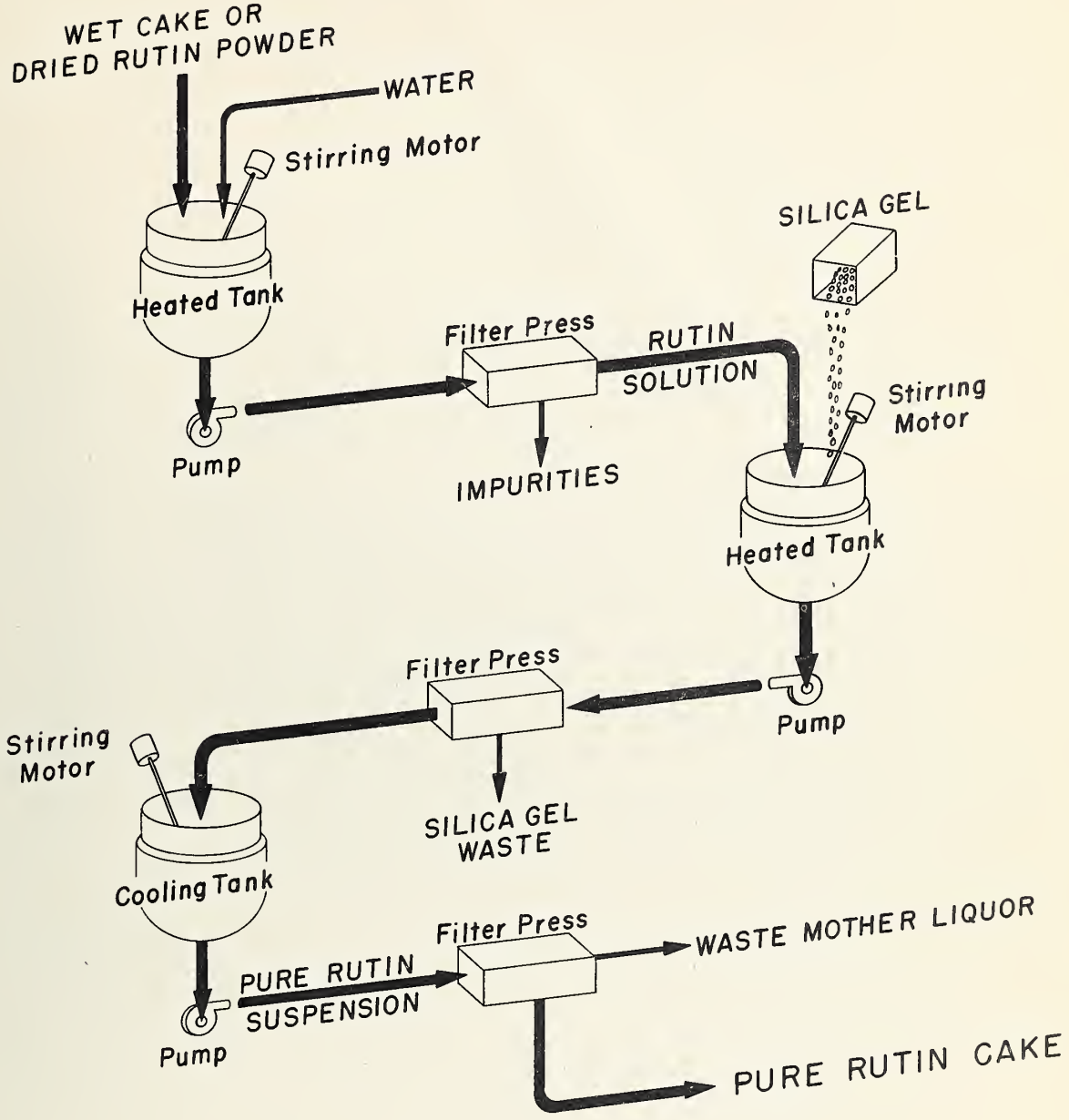
This section is designed to describe the various published reports concerning the pharmacology of rutin and to furnish a brief general review of existing knowledge in this field. Although many of the reports are conflicting and contradictory, no attempt has been made to evaluate them critically since such evaluation is beyond the scope of the present work.

[2] Couch, J. F., Krewson, C. F., and Porter, W. L. U. S. Patent No. 2,500,930. March 21, 1950.

PRODUCTION OF RUTIN FROM BUCKWHEAT



REFINING OF CRUDE RUTIN



TOXICITY

The experience of all investigators who have studied the pharmacology of the flavone derivatives has indicated their lack of toxicity. Indeed the failure of these substances to produce apparent symptoms in animals resulted, with a few exceptions, in neglect of the study of the entire group as physiological agents until recent years.

The most extensive investigation of the toxicity of rutin was carried out by Wilson, Mortarotti and Doxtader (136). Intravenous and intraperitoneal injections of 30 to 50 mg/kg. of rutin in rats and guinea pigs and intravenous injections of 100 to 200 mg/kg. in rabbits were innocuous. The rate of growth of albino rats was not affected by a diet containing 1 per cent of rutin. After 400 days on this diet, histological examination of the tissues showed no evidence of injury that could be related to rutin administration. The organ weights of the experimental animals were normal.

A series of prolonged feedings to guinea pigs was conducted under the supervision of L. T. Giltner[3]. The animals were fed a daily ration containing 10 or 20 mg. of rutin for 8 weeks. At the end of that time they were sacrificed and autopsied. The post mortem findings were normal. The animals made normal gains in weight during the experiment, and at no time were any abnormal symptoms noted.

In the course of their study of the excretion of rutin by man, Porter, Dickel and Couch (103) administered rutin to three healthy subjects in doses of 2.25 grams per day for 7 days. No symptoms of any kind resulted.

Similar statements concerning the lack of toxicity of rutin were made by Brandl and Schartel (15), Garino (52), Mascre and Paris (93) and Czimmer (35).

The clinical experience of physicians who have had patients on rutin for prolonged periods of time indicates that the drug is nontoxic to man. Some of the patients have been on rutin for upwards of 5 years, with a daily intake of 60 mg. or more. All these data furnish convincing proof that rutin is innocuous.

ABSORPTION AND EXCRETION

In 1913 Garino (52) administered rutin, quercitrin, hesperidin and naringin by mouth and intravenously in comparatively large doses to dogs, and recovered rutin and hesperidin unchanged from the urine. His data indicate that a large portion of the flavonoid is quickly excreted.

Kono (78) studied the excretion of rutin and several other flavonoids by the kidney and liver in rabbits. He reports that 50 to 90 percent of the intravenously injected flavonol was excreted by the kidney in 1 hour but that when a choleduct canula was employed the greater part of the flavonol (30-70 percent) was excreted by the liver in 2 hours and less (20-47 percent) by the kidney.

[3] Unpublished data obtained at the Eastern Regional Research Laboratory.

In a second paper (77) Kono studied the absorption of flavonols from the gastrointestinal tract and also after subcutaneous injection and concluded that rutin and other flavonoids are rapidly absorbed from the subcutaneous tissues and appear in the urine nearly as rapidly as after intravenous injection.

In the course of developing a reliable method for the determination of rutin and other flavonols in urine, Porter et al. (103) obtained some data concerning the absorption and excretion of rutin in man. Using a spectroscopic method, these workers found that often only very small quantities of rutin appeared in the urine even after doses as large as 2.25 g. per diem continued for 7 days. Tests of the feces did not reveal the presence of flavonols, so that one must conclude either that rutin is stored in the tissues or is rapidly metabolized to products not detectable by the technique employed.

When administered intravenously, however, a fraction of the rutin appears in the urine. Rabbits were severally given rutin by mouth and intravenously by Dr. F. DeEds, and a 24-hour specimen of urine was collected. In the samples obtained from rabbits given rutin by mouth, only traces at most of rutin could be detected. In the intravenous cases, one-eighth of the injected rutin was found in the urine. Clark and Mackay (143) have recently confirmed these findings and have extended them to include several other flavonoids.

BLOOD PRESSURE EXPERIMENTS

Mascré and Paris (93) studied the action of rutin injected intravenously into the chloralosed dog. A 0.1-percent solution of rutin in 30 percent alcohol caused "phenomena of hypertension" with bradycardia, accompanied by a diminution in the volume of the kidney.

Injection with a 10 percent solution of rutin in 10 percent pyridine in doses of 1 to 5 mg./kg. caused a lowering of blood pressure of 3 to 5 cm. of mercury which persisted for 3 to 4 minutes.[4]

Fukuda (50), however, reported that rutin and other flavonols caused a risel in blood pressure in rabbits and frogs, which he attributed to a contraction of the blood vessels, an increase in heart action, and hydremia.

Czimmer (35) observed that rutin did not affect the blood pressure of the cat.

Armentano (6) administered a number of flavonoids to dogs and cats intravenously and studied the effects on the blood pressure. Rutin was not used. Quercitrin, quercetin, rhamnetin, citrin, naringenin were active in reducing blood pressure, quercitrin being the most active.

ASCORBIC ACID SPARING EFFECT

The apparent ability of certain flavonoid compounds to supplement the action of ascorbic acid was early noted by Szent-Gyorgyi's group (13). Guinea pigs on a scrobutic diet did not develop scurvy when "citrin", hesperidin, or

4 JOHN Q. GRIFFITH, JR., M. D. COMMENTS THAT A FALL IN BLOOD PRESSURE OF 30 TO 50 MM. IN A NORMOTENSIVE DOG COULD RESULT IN FATAL SHOCK. (PERSONAL COMMUNICATION).

demethylohesperidin was added to the diet. Quercitrin, however, failed to prevent scurvy when fed with the scorbutic diet. The authors reasoned from these data that the presence of the unsaturated linkage between C atoms 2 and 3 and the -OH group on No. 3 "entails inactivation."

Papageorge and Mitchell (99) reported an increase in the concentration of ascorbic acid in the adrenals of guinea pigs on an adequate intake of vitamin C supplemented with rutin and suggested that the antioxidant action of rutin especially on epinephrin, whose oxidation products contribute to the oxidation of ascorbic acid (67), results in a sparing action on this acid. Papageorge, Noble, and Amerson were (163) unable to repeat these results on adrenal ascorbic acid.

Crampton and Lloyd (32), using the odontoblast method of assay, studied the effect of rutin on the biological potency of vitamin C, and found that the rutin treatment gave significantly higher values on the response-dose curve at three levels of ascorbic acid. They state: "At the lower levels of vitamin intake, it appears that rutin either makes more available or delays *in vivo* destruction of ascorbic acid in the original source."

Ambrose and DeEds (138) made an extensive investigation of the possible supplementary or sparing action of rutin or quercetin on ascorbic acid given in subminimal doses to guinea pigs on a scorbutogenic diet. Rutin or quercetin could not be substituted for ascorbic acid in the diet. Animals so treated developed scurvy and ran a course not different from that of the controls. The doses used were 100 mg. of rutin or 50 mg. of quercetin daily administered orally in propylene glycol solution. Animals given 0.2 mg. of ascorbic acid and 100 mg. of rutin or 50 mg. of quercetin daily did better than the controls on 0.2 mg. of ascorbic acid alone. Their general appearance was better, symptoms were less evident, and joint swelling and stiffness were less frequent. The life of guinea pigs receiving ascorbic acid and rutin was prolonged beyond that of those receiving ascorbic acid, rutin or quercetin alone. The post-mortem picture was essentially the same for all groups except that fewer fresh hemorrhages were observed in the groups that received the flavonols and subminimal doses of ascorbic acid. Continuing their investigations, Ambrose and DeEds (139) were able to demonstrate that the quantity of adrenal ascorbic was independent of the intake of rutin which plays no important role as an "economizing factor" on the storage of adrenal ascorbic acid under the conditions of their experiments.

RADIATION INJURY

In 1947 Griffith, Anthony, Pendergrass and Perryman (57), observing that one of the effects of excessive radiation appeared to be an increase in capillary fragility, investigated the effect of rutin treatment on the radiation reaction in rats (58). All animals were given radiation to one leg, 2385 r. in a single dose. At the same time, in half the animals a pellet of 20 mg. of rutin was implanted along the lateral aspect of the abdominal wall, and this was repeated every third day for 36 days. There was no significant difference between the time of onset of the reaction between the two groups. Between the twenty-first and twenty-fifth day, 12 of the rutin-treated animals became normal, as against one of the control group. On the thirty-fifth day, 11 of the controls and 2 of the rutin-treated animals still showed an abnormal foot. Rutin appeared to have hastened the recovery time after irradiation.

Control of the hemorrhagic syndrome and reduction of x-irradiation mortality in dogs was reported by Rekers and Field (108). Two groups of 25 similar dogs were given a single application of total-body x-irradiation of midle-thal dose, 350 r. One group was given 50 mg. of rutin t.i.d. beginning 1 week prior to the irradiation and continuing through the experiment. The other group served as controls. Sixteen of the control group succumbed in 13 - 30 days after x-irradiation; only 3 of the rutin-treated died -- 16, 28 and 31 days post radiation. The controls that died showed widespread ecchy-moses and intrapulmonary and intra-intestinal hemorrhages. Characteristic widespread hemorrhage occurred in 2 of the 3 rutin-treated dogs that died; the remaining 22 dogs were relatively free from petechiae and ecchymoses during the post-irradiation period (40-60 days) and at autopsy. Three of the surviving controls manifested subcutaneous ecchymoses and intestinal hemorrhages. Several of the rutin-treated dogs developed severe thrombo-cytopenia and leucopenia, which persisted from 10-14 days, with eventual re-covery. Untreated dogs rarely recovered under these conditions.

In more extensive reports (44, 45, 46), Field and Rekers presented the re-sults of their study of a variety of vitamin P substances, rutin, hesperidin, hesperidin methyl-chalcone, esculin and a lemon peel preparation. With mid-lethal doses of x-radiation, hesperidin and the lemon peel preparation af-forded some protection but less than rutin. The methyl-chalcone, esculin, and vitamin C did not. Rats given 700-750 r. were not protected. When a full lethal dose of radiation was given (450 r.), rutin therapy failed to provide a detectable reduction in clinical symptoms or mortality.

Kohn, Robinett and Cupp (76) studied the effect of x-irradiation on rats treated with rutin and found no effect upon the L.D. 50, the survival fol-lowing two sublethal exposures, the coagulation time, the thrombocyte count, the total white cell count, and the hemorrhage into the lymph nodes and G.I. tract. Rutin did not affect the clotting time of normal human or rat blood, nor did it prevent the action of heparin. It did antagonize the antiheparin action of toluidine blue, preventing clotting when present in an equal quantity by weight.

Cronkeit and co-workers (33), working with mice, were unable to obtain pro-tection from x-irradiation when the animals were given rutin. Detailed dis-cussions of the effects, particularly of "atomic" radiation, on man and animals are given by Cronkeit (144) and Cronkeit and Chapman (145).

Clark, Uncapher and Jordan (21) used a water-soluble preparation from lemon peel termed "calcium flavonate" to treat guinea pigs exposed to 220-225 r., a dose that consistently killed 67 percent of the control animals, 50 per-cent dying within 13 days. Only 35 percent of the treated animals died, none dying within 13 days. The hemorrhagic symptoms of the treated animals were considerably less marked than those of the controls.

Haley and Harris (64) have studied the hematological response of guinea pigs to x-irradiation and found a significant increase in the clotting time of the blood, probably correlated with a decreased number of platelets.

Sokoloff, Redd and Dutcher (165) exposed rats of the British brown breed to 800 r. of total body radiation in one exposure. Rats "protected" with a citrus-derived vitamin P preparation showed a much lower mortality than the controls.

EPINEPHRIN

In 1941 Lavollay and Neumann (82) published the results of their early researches on the "vitamin P function," undertaken at the instance of Maurice Javillier. Assuming that the property of controlling capillary permeability could be "attributed to an effect on the tonus of the precapillary vessels" and that this effect could be "manifested through the intervention of sympathin," they sought evidence of "preservation of sympathin" by vitamin P. Purified extracts of oranges, free of hesperidin, strongly inhibited the oxidation of epinephrin *in vitro*. Since they suspected the presence of flavonoids in citrus extracts, the French workers tested the effect of some of these substances on the autoxidation of epinephrin. Quercitrin, rutin, and naringin were found inhibitory in decreasing order of activity.

The chemical evidence was supported by pharmacological data. Extract of oranges and the flavonol derivatives prolonged the action of epinephrin on the isolated guinea pig intestine and seminal vesicle. In the dog, a preliminary intravenous injection of quercitrin prolonged the effect of an injection of epinephrin and eliminated symptoms of shock following an injection of peptone.

Later in the same year, Lavollay published a second note (81), presenting data on the antioxidant action of certain flavonoids toward epinephrin. There appeared to be no chemical structure common to all these substances to which the capillary action could be attributed. Javillier and Lavollay (70) have summarized the researches of the French school in some detail.

The epinephrin "sparing" action of rutin was studied by Wilson, Mortarotti and DeEds (135), who found a definite prolongation of the epinephrin effect on the isolated guinea pig colon following addition of rutin to the fluid bathing the intestinal strip. Clark and Geissman (18) developed a method of assay based on the inhibition of oxidation of epinephrin by which they studied a number of orthodihydroxyphenolic compounds and metal-complexing agents. They reported that the ortho hydroxy groupings must be free, since methylated derivatives like hesperidin are nearly devoid of activity. They suggest that activity is probably related to the reducing powers of the compounds, quinone formation, and metal-complexing or chelating capacity, and conclude that it is doubtful that epinephrin antioxidant properties and capillary fragility (permeability, filtration) are exclusively related so far as vitamin P effects are concerned. These authors published a more extensive summary (19) and complete data (20), in which they present results obtained with 68 compounds and calculate the quantitative epinephrin potentiating activities of the various substances. About 17 compounds showed greater potentiating activity than rutin.

Wilson and DeEds (133) found that copper in similar experiments decreased the epinephrin half-recovery times. In this work, the authors made several contributions to our knowledge of the relationship between chemical constitution and epinephrin-protecting action. A glycosidal linkage at the C-3

position does not modify activity, at the C-7 linkage it increases activity, and methylation of the C-7 hydroxyl decreases activity. Activity is increased by a double bond between C-2 and C-3. Free ortho hydroxyls on the phenyl ring also increase activity.

The antioxidant theory has recently been criticized on physiological grounds by Sokoloff and Redd (121, 122) in these terms: "The leading idea in Lavollay's work is that vitamin P factors delay oxidation of adrenalin and that their usefulness as far as capillary permeability is concerned is ended. In other words, vitamin P factors have no direct effect upon either the capillary wall or capillary permeability. And in every one of their observations these workers have tried to find new evidence to support their fundamental theory. Not only have they not proved that adrenalin maintains capillary tone; not only were they unable to demonstrate that adrenalin increases capillary resistance or decreases capillary permeability, but they have offered an insufficient evidence in defense of their basic viewpoint about the delaying action of vitamin P on oxidation of adrenalin. It is true that Parrot and Cotereau have proved that vitamin P factors delay *in vitro* oxidation of ascorbic acid and adrenalin. But no evidence has been offered by them so far that vitamin P factors act in the same manner *in vivo*.

"There is not sufficient evidence available as to the connection between the vaso-constrictive effect of adrenalin upon blood vessels and the decrease of capillary fragility."

HISTAMINE AND ANAPHYLACTIC SHOCK

Wilson, Mortarotti and DeEds (133, 135) found that, under certain specific circumstances, rutin injected intraperitoneally in 10-mg. doses would protect guinea pigs fed on a vitamin P deficient diet against an LD 50 dose of histamine dihydrochloride. When the rutin was given 10 to 30 minutes prior to the intravenous injection of histamine, considerable protection was afforded. This did not occur if the rutin was given earlier, 35 to 65 minutes before the histamine. Rutin and histamine injected simultaneously resulted in no protection to the animals, indicating that there is no direct antagonism between the two substances.

Hiramatsu (68) reported protection of guinea pigs against anaphylactic shock by pretreating the animals with vitamin P. In a preliminary study which was not reported in detail because the data are too meager, Wilson, Mortarotti and DeEds (135) were unable to confirm this finding.

Raiman, Later and Necheles (105) sensitized guinea pigs by an intraperitoneal injection of 0.25 ml. of normal horse serum. After 12 days, the animals were shocked by a parenteral dose of horse serum. Animals given rutin intraperitoneally 30-45 minutes before the shocking dose exhibited no symptoms of shock, whereas the control animals were all dead in 10 minutes. One rutinized animal that received the rutin dose 60 minutes prior to the shocking dose died in anaphylactic shock in about 15 minutes.

Guinea pigs given a lethal dose of histamine 30 to 45 minutes after receiving 1 mg. of rutin intraperitoneally died of histamine shock within 10 minutes. Wilson and DeEds (134) have commented on these results. Clark and Mackay (142) obtained a slightly diminished histamine diphosphate toxicity after rutin or the sodium salt of quercetin sulfonic acid. Several other flavonoids were inactive.

Levitan (85) pretreated rabbits with large doses of rutin, up to 2 g., and failed to obtain protection against a minimum lethal dose of histamine. He also was unable to inhibit sensitization to horse-serum and to suppress the anaphylactic reaction in the rabbit with large doses of rutin.

Arjona and his co-workers (167) were unable to protect guinea pigs against anaphylactic shock with rutin.

Hepding (152) was unable to protect guinea pigs against histamine shock with rutin.

Roth and Shepperd (112) studied the protective action of rutin against shock due to horse serum. Guinea pigs given 1 to 20 mg. of rutin intraperitoneally were not protected. There was little protection noted against egg white under the same circumstances. When an LD 100 dose of histamine was given to guinea pigs, the authors could observe no protection on 10-mg. doses of rutin.

DIURESIS

The question of a possible diuretic effect of flavonols has engaged the attention of several workers in this field. Akamatsu (2) administered rutin to rabbits in doses of 0.3 to 0.5 g./kg. and observed a significant increase in the volume of urine excreted daily. The increase was observed as long as rutin was administered. When rutin was withdrawn, the volume of urine returned to normal.

Akamatsu also reports that there is a synergism between rutin and the purines, caffein and theophylline. Combinations of rutin with caffein or theophylline exert a more intensive and prolonged diuresis than either drug alone.

At the same time Fukuda and Kono (51) studied the diuretic action of several members of the flavonol group, including rutin, on rabbits. All the substances examined were "active diuretics".

Mascré and Paris (93) obtained contrary results with dogs.

Czimmer (35) reported diuresis in rats following injection of rutin solutions but not in frogs, guinea pigs, rabbits, or cats. Carnivorous animals excreted in the urine a substance that gave a green color with ferric chloride. This was absent or doubtful with herbivorous species.

BACTERIOSTATIC ACTIVITY

In a study of the effect of added flavonoids on the bacteriostatic action of dicumarol, Naghski, Copley and Couch (96) observed that not only did the glycosides rutin and quercitrin and the aglycone quercetin neutralize the

bacteriostatic properties of dicumarol but that quercetin itself possessed considerable toxicity toward *Staphylococcus aureus*, completely inhibiting growth in a concentration of 0.1 mg./ml. Rutin was without bacteriostatic action, but quercitrin exhibited a slight toxicity toward the organism. This effect may have been due to a small quantity of quercetin formed by partial hydrolysis of the rhamnoside. However, since the rutin used is now known to have contained 2-3 percent of quercetin, the probability is that the bacteriostasis observed was due to the rhamnoside itself. Up to this time it was not known that the flavonols possess any bacteriostatic properties.

Later in the same year Andersen and Berry (5) published the results of their studies of the effects of rutin, quercitrin and quercetin on toxin formation by *Clostridium botulinum* types A and B, in a medium of green peas and corn steep casein. Quercetin in concentrations of 80 to 160 p.p.m. prevented toxicity from developing in the medium. Rutin was ineffective, and quercitrin showed little activity in concentrations of about 1000 p.p.m. Quercetin, however, was not able to inactivate perform botulinus toxin.

Ma and Fontaine (87) reported that rutin and quercetin exert an antagonistic effect on the antibiotic activity of crystalline tomatine toward *Candida albicans*. The inhibitory effect of tomatine (0.1 mg./ml.) was counteracted by rutin (0.2 mg./ml.) and quercetin (0.5 mg./ml.). Tomatine 0.25 mg./ml. required 0.5 mg./ml. of quercetin and rutin had no effect at 1 mg./ml. The flavonols by themselves had no effect on the growth of the organism.

Bustinza and Lopez (16) found that quercetin inhibits the growth of *Staphylococcus aureus, B. mycoides, Mycobact. phlei, M. smegmatis, M. avium* and *M. tuberc. hominis*.

Naghski, Copley and Couch (97) found that the antibacterial activity of quercetin was most pronounced below pH 7 and negligible above that figure. At pH 6.5 and in concentrations of 0.075 to 0.10 mg./ml., quercetin produced complete inhibition of *Staphylococcus aureus, S. albus, Aerobacillus polymixa,* and *Brucella abortus*. Partial inhibition was obtained of a strain of group D and group E streptococcus and several gram-negative organisms. *Mucor racemosus* was inhibited 25 to 30 percent by 0.15 mg./ml., but 5 other molds were not affected. The activity of quercetin was lost in the presence of serum and iron but was not affected by cysteine. Rutin and quercitrin were inactive.

ANTIVIRAL EFFECTS

Cutting, Dreisbach and Neff (34) studied the prophylactic action of several flavonoids against fixed rabies virus in mice. The virus was inoculated intra-cerebrally 4 days after administration of the drug was begun, and the drug was continued until the end of the experiment. Of the substances tried, quercetin and quercitrin showed significant prophylactic activity. Rutin gave promising results; -- 14 of the animals survived as against only 4 of 21 controls -- but the number of animals used was considered too small for statistical treatment.

FROSTBITE

The work of Fuhrman and Crismon (49) has demonstrated the value of rutin therapy in experimental frostbite in rabbits. In 15 control animals, immersion of the foot to the level of the tuberosity on the fifth metatarsal in liquid at -55° C. resulted in complete loss of the exposed part in 11 cases and loss of all but a narrow portion of the plantar pad in 4 cases. Of 10 animals treated with rutin, 50 to 100 mg. per diem orally, 9 lost only toes and 1 lost toes plus about 1 cm. of foot. Rutin was not effective in preventing loss of tissue after frostbite of rabbit ears. Rutin-treated animals did not develop stasis in the true capillaries of frostbitten ears as early as did untreated animals.

Ambrose, Robbins and DeEds (4) have confirmed the results reported by Fuhrman and Crismon and have shown that some degree of protection is afforded by other flavonoids -- quercitrin, quercetin, methyl hesperidin chalcone, 2-3 dihydroquercetin and an extract of muscat raisin seeds.

HYALURONIDASE

Levitan (84, 86) states: "Rutin and esculin, two types of vitamin P, inhibited the spread of intradermally injected hyaluronidase, azoserum and saline."

Elster (41), however, was unable to confirm Levitan, using white rats given rutin 200 mg. by stomach tubes or intraperitoneally in propylene glycol or saline for 4 days prior to operation.

Beiler and Martin (10) reported that rutin, *inter alia*, inhibited the action of hyaluronidase in concentrations of 1 mg. per cc. When rutin was combined with ascorbic acid, this action was much enhanced.

HISTIDINE DECARBOXYLASE AND CHOLINE ACETYLASE

Martin and co-workers (159) tested the inhibitory action of vitamin P compounds on histidine decarboxylase and found rutin and esculin inactive, whereas the aglycons, quercetin and esculetin, were active. Beiler and coworkers (11) have studied the inhibiting action of a number of flavonoids on acetylcholinase. Compounds that can form 3-4 diketones appear more active than others. Thus quercetin and esculetin are much more active than their glycosides rutin and esculin.

ANTAGONISM TO CAPILLARY EFFECTS OF CERTAIN DRUGS

Richards and Kueter (109, 110) reported antagonism of rutin to the capillary effects of sodium bisulfite and procain. Sodium bisulfite increases the toxicity of epinephrin and procain given subcutaneously or intramuscularly but after intravenous injection of rutin the increase is greatly reduced The toxicity of procain alone is also reduced, as is that of strychnine. Haley and Rhodes (150) used this observation as a basis for a method for determining capillary permeability. They tested a number of flavonoids by this technique and report the results. It is doubtful, however, whether what they were measuring is correlated with "vitamin P" activity.

Working with rats, Clark (17) was unable to demonstrate any antagonism between rutin and dicumarol.

Martin and Swayne (92) also working with rats, however, found evidence of antagonism between dicumarol and 2 flavonoids, rutin and d-catechin, and also ascorbic acid. Hesperidin did not antagonize dicumarol. A synergism between ascorbic acid and d-catechin was observed which was found also in antihyaluronidase action (10) and in the antioxidant action on epinephrin (107) when rutin was present in the system.

EFFECT ON COAGULATION OF BLOOD

Plungian, Munch and Wolffe (101) studied the effects of rutin, bile salts, and dicumarol separately and together on the coagulation time of white rat's blood. Rutin, orally, and bile salts, decreased the coagulation time of the blood, but dicumarol (6 mg.) increased it.

Kohn, Robinett and Cupp (76) studying the effects of irradiation on white rats were unable to obtain evidence that rutin affected the normal clotting time of human or rat bloods.

Field and Rekers (44) found that the clotting time of dogs' blood after irradiation, with or without rutin, was not markedly increased.

ALKALI RESERVE

Lecoq (83) studied the effect of rutin and d-epicatechin on the alkaline reserve of the blood plasma when given to rabbits intravenously. Doses of 0.3 mg. of rutin and 10 mg. of epicatechin increased the alkali reserve. Vitamin K did not increase the reserve.

CAPILLARY DILATION

Haley, Clark, and Geissman (63), using the rat meso-appendix preparation of Chambers and Zweifach, studied the effect of several flavonoids on capillary vasomotion. They found rutin and its acid succinate inactive, the rutin acid phthalate slightly active, and the catechins very active. Czimmer (35) found that rutin caused a contraction of the frog capillaries. Sokoray and Czimmer (123) reported vasoconstriction in certain perfusion experiments with quercitrin. Fuhrman and Crismon (49) however, considered it doubtful that peripheral vasoconstriction involving arterioles could be a factor in the protection of rabbit's feet from the effects of frostbite.

CANINE HEMORRHAGE

Hellerstein et al. (66) studied the effect of rutin on the hemorrhagic phenomena of experimental malignant hypertension in the dog. Acute hypertension with uremia was produced in 16 dogs by bilateral ligation of the renal arteries. All the animals developed clinical uremia and hypertension. and died in 3 to 6 days. Severe hemorrhages in the gastrointestinal tract,

heart, pancreas, urinary bladder, diaphragm, spleen and adrenals, together with myocardial inflammation and necrosis occurred in control dogs and those given 200 mg. of rutin postoperatively and for 3 days preoperatively. Dogs given rutin for 10 days preoperatively showed complete absence of cardiomyopathy and of the hemorrhagic changes. The authors think that contradictory reports on the action of rutin in hypertension may be due to the relative deficiency of rutin in the patients studied.

SHWARTZMAN PHENOMENON

The influence of several antihistamine and flavonoid compounds upon the Schwartzman reaction in which there is a markedly increased capillary fragility was studied by Maratka and Ivy (90). Rutin, hesperidin and citrin inhibited the phenomenon.

CARDIAC EFFECTS

Akamatsu (1) tested the action of rutin and four other flavonoids on the isolated and *in situ* frog heart. In all cases, the amplitude of the heart beat was increased, the pulse rate diminished, and the minute volume increased. Von Jeney and Czimmer (71) reported that quercetin and quercitrin had a slightly increasing action on the healthy frog heart. They antagonized the toxic action of chloroform, urethan and quinine HCl on the frog heart. Rhamnetin had a similar action (72). Hesperidin depressed the heart action and did not antagonize the depressive effect of lactic acid. E. Jeney (74) found that quercetin and quercitrin antagonized the toxic action of methyl alcohol on the frog heart. Rhamnetin was much weaker. Von Jeney, Mehes, Czimmer and Sokoray (73) obtained irregular and contradictory data when they attempted to repeat these experiments on mammals, especially the guinea pig. The action on the heart was sometimes toxic and at other times depressant, and in about half the cases no effect was noticed. They used quercetin, quercitrin and forsythia extract (rutin). Czimmer (35) reported that the forsythia glycoside (rutin) acted on the frog heart like other flavonols. DeEds (37) obtained definite stimulation of the frog heart with rutin.

CLINICAL APPLICATIONS

INCREASED CAPILLARY FRAGILITY

The use of rutin in correcting increased capillary fragility was announced in 1943 by Griffith, Lindauer and Couch (61). Rutin was administered to 14 patients with hypertension complicated by increased capillary fragility as determined by the positive pressure method of Göthlin (54). Eleven of these patients were followed for 12 to 16 months. In 8, the capillary fragility became normal within 2 months; it remained increased in the other 3, 1 of whom developed a hemiplegia 4 months after beginning medication. No complications developed in the other 10 patients during the period of observation. Two subjects stopped taking rutin after the capillary fragility had returned to normal, and within 6 weeks the fragility was again increased. When rutin was resumed, the fragility returned to normal within a month (59).

In succeeding papers Griffith (55) and Griffith and Lindauer (60) reported results with a more extensive series of patients which indicated that 88 percent of patients with an initial capillary fault returned to normal after rutin medication. The tendency to retinal hemorrhage or apoplexy, more pronounced in hypertensive patients with capillary fault, is reduced after the fault is corrected by rutin to a figure comparable with that found in patients without capillary fault.

Statistics of the patients treated are presented in Tables 1 and 2[5].

Similar results have been reported by Shanno (118), Zfass (137), Hein (65), and Gutierrez (62). Donegan and Thomas (39) observed improvement following rutin therapy, although the index did not return to a normal level. Diabetics with retinopathy were more refractory, but hypertensives reacted favorably, and of 61 cases with retinopathy, 43 showed improvement in this condition following rutin therapy. Eight of 9 cases of Eales's disease were improved, the capillary fragility returning nearly to normal.

McManus and Landrigan (94), using a suction method to determine capillary fragility, treated 10 patients with rutin for 4 weeks while 5 other patients were kept as controls. Rutin was given 40 mg. t.i.d. to one-half and 20 mg. t.i.d. to the other half of the treated patients. Suction at -20 cm. of mercury was applied for 1 minute. There was considerable variation in the number of petechiae that occurred throughout the period of observation. There was an average decrease of 12 petechiae in the 40-mg. group and 11 in the 20-mg. group. The control group registered an average decrease of 8 petechiae during the same period. The authors conclude that in the dosage used rutin given for 4 weeks was ineffective in decreasing capillary fragility in the 10 patients.

Schweppe, Lindberg and Barker (117) administered rutin to 7 patients with increased fragility. In 3 cases, fragility returned to normal. In 3 others it remained border line, and in the seventh, after an initial improvement during the first month, it again became abnormal in the second month in spite of continued treatment.

Koenigsberg (153) reported good results in reducing increased capillary fragility by rutin in hypertensive cases.

[5] These tables are taken from Charts 8 and 10 published in a processed brochure (Jan. 1949) compiled by J. Q. Griffith, Jr., M. D. and J. F. Couch and reproducing a series of charts exhibited before the American Medical Association in June 1948, and other medical societies at various times. The statistics were furnished by J. Q. Griffith, Jr., M. D. and M. A. Lindauer, M. D. The brochure contains contributions from 23 physicians, pharmacologists and chemists working in the field. Copies may be obtained gratis from the Eastern Regional Research Laboratory, Philadelphia 18, Pennsylvania.

TABLE I

INCIDENCE OF COMPLICATIONS IN PATIENTS TREATED FOR CAPILLARY FAULT.

AVERAGE FOLLOW UP, 16 MONTHS

CAPILLARY FRAGILITY AND/OR CUTANEOUS LYMPHATIC FLOW	INCREASED AT START (450 PATIENTS)		
	APOPLEXY	RETINAL HEMORRHAGE	DEATH
RESTORED TO NORMAL	7(1.5%)	2(0.4%)	14(3.1%)
REMAINED INCREASED	19	12	13
UNDETERMINED	15[1]	3	26[2]
TOTAL	41(9.1%)	17(3.8%)	53(11.8%)

CAPILLARY FRAGILITY AND/OR CUTANEOUS LYMPHATIC FLOW	NORMAL AT START (361 PATIENTS)		
	APOPLEXY	RETINAL HEMORRHAGE	DEATH
NORMAL	5(1.4%)	3(0.8%)	13(3.6%)

[1] *8 not on rutin*

[2] *17 not on rutin*

TABLE 2

RELATION BETWEEN THIOCYANATE THERAPY AND CAPILLARY ABNORMALITY

EFFECT OF RUTIN THERAPY

GROUP NUMBER	PREVIOUS THERAPY	CAPILLARY FRAGILITY	CUTANEOUS LYMPHATIC FLOW	THERAPY SCN OR RUTIN	NUMBER OF SUBJECTS WITH REPEATED TESTS*	FOLLOWING THERAPY (COLUMN 6) PER CENT OF PATIENTS WITH REPEATED TESTS INCREASED	NORMAL
1	NONE	NORMAL	NORMAL	NONE	59 44	0 0	100% 100%
2	NONE	NORMAL	NORMAL	SCN	88 36	18% 17%	82% 83%
3	RUTIN(a)	NORMAL	NORMAL	SCN	62 21	18% 19%	82% 81%
4	SCN(b)	INCREASED	INCREASED	STOP SCN	6 1	0 0	100% 100%
5	SCN(b)	INCREASED	INCREASED	RUTIN BEGUN OR INCREASED	20 9	0 0	100% 100%

* Repeated tests were always at least 6 weeks apart. The average number of repeated tests per patient for groups 1, 2 and 3 was 2.7.

(a) These patients had, initially, increase in either fragility or lymphatic flow or both, but these returned to normal following rutin therapy.

(b) From Groups 2 and 3.

Paris and Vairel (168) studied the influence of rutin and several other flavonoids on the capillary resistance and permeability of the rabbit. Rutin, scoparin, quercitrin, quercetin, luteolin and catechin were active.

Mathiesen (160) gave rutin 20-40 mg. t.i.d. to 7 patients with increased capillary fragility. Four responded well, 1 was doubtful and 2, 1 of whom had glomerulonephritis, did not respond.

Muschaweck (162) reported increase in capillary resistance of white rats following therapy with rutin, mono-, di-, and trimethylolrutin but not after the boric and succinic esters of rutin.

Hepding (152) confirmed the protective action of rutin on capillary integrity in guinea-pigs.

A comparison of results obtained by the Göthlin and Rumpel-Leeds techniques on 50 patients who had been treated with full doses of ascorbic acid for a month or more prior to the test was reported by Soloff and Bello (124). The patients were hypertensives complicated with ocular disorders, many with hemorrhages. The results of the comparison are very interesting. Of the 50 patients, only 2 showed increased capillary fragility by the Göthlin test. One was diabetic and the other suffered with Paget's disease. The Rumpel-Leeds test indicated that 33 of the patients were positive, 14 of whom had a "countless" number of petechiae. Ten patients with positive R-L tests were treated with rutin, 60 to 80 mg. per diem for 1 month, without reversal of the test.

PURPURA

The use of rutin to correct the vascular fragility characteristic of non-thrombocytopenic purpura was investigated by Madison and Belfus (89). Preliminary studies with 14 patients gave encouraging results. In 21 cases of clinical purpura observed over an extended period of time, rutin produced reversal of vascular fragility in 1 case, marked improvement in 3 cases, moderate improvement in 10 cases, and no change in 7 cases. In 13 of the 21 cases there was subjective improvement, as manifested by the diminished numbers of bleeding petechiae and ecchymoses and desire or willingness of the patient to continue the drug.

Belfus and Madison (12) produced experimental purpura in guinea pigs with antiplatelet serum. Animals to which 10 mg./kg. of rutin was given showed a more rapid reduction in the number of petechiae than the controls. The red cell count and the platelet count were but slightly increased over the controls.

Copely (23) reported the successful treatment of three cases of nonthrombocytopenic purpura with rutin. His treatment is of interest in that he controlled the progress of the disease by frequent use of his ecchymoses test and increased the dose of rutin until the appearance of ecchymoses became doubtful. In one case the dose was increased to 600 mg. per diem. In the other two it was finally increased to 1000 mg. The high dose was continued for 5 days in one instance and for 10 days in the other. No ill effects from these large doses of rutin were observed.

Doumer et al. (40) reported a case of purpura with lowering of capillary resistance, which was improved by treatment with rutin and adrenochrome.

Randall and Sevringhaus (106) have reported a careful study of the effects of a number of flavonoid compounds on capillary resistance in thrombocytopenic purpura of rats, produced by antiplatelet serum. Rutin given orally was not so effective in affording protection as certain others which were, however, given intravenously.

TELANGIECTASIA

Hereditary hemorrhagic telangiectasia or Osler's disease is a condition in which rutin should *a priori* be beneficial. Several cases have been reported in which rutin has been effective in controlling the gastrointestinal hemorrhages and the nose and gum bleeding, even when these had been present for long periods.

S. D. Kushlan (80) was the pioneer in this application of rutin. In 1946 he reported the successful use of the glycoside in a case of Osler's disease of 40 years' standing. The patient had been under observation for 3 years when rutin became available for use. Within 24 hours after treatment with rutin, 40 mg. t.i.d. orally, was begun the daily epistaxes and bleeding from the gums ceased for the first time since childhood, and had not recurred. Occult blood in the stools gradually diminished, and disappeared on the sixteenth day after rutin was begun. The dose was reduced to 20 mg. t.i.d. ferrous sulfate 6 gr. t.i.d. being given to correct the serious anemia. The patient was able to return to work 1 week later "feeling better than ever, physically and psychologically."

An interesting sequel to this case was reported by Dr. Kushlan. The same patient was given sulfadiazine by his urologist for an infection of the urinary tract. The nosebleeds returned, and there was evidence of major gastrointestinal hemorrhage. The sulfa drug was discontinued, and the dose of rutin doubled. The bleeding promptly ceased, with no recurrence at the time of writing.

Other cases of telangiectasia successfully treated with rutin have been reported by Cope and Grover (22), Markoff (91), Schwartz and Armstrong (116), Springer and Shannon (125), Rumball (113) and Gambacorta (148).

IDIOPATHIC PULMONARY BLEEDING

Two cases of this type are presented by Shanno (118). A 38-year-old housewife complained of bleeding from the mouth for 3 months. This occurred 3 to 4 times a week and amounted to about 1 drachm at a time. Examination revealed no evident cause. The Gothlin Index was 17, definitely abnormal. Rutin was given, 20 mg. t.i.d. At the end of 3 weeks the bleeding had ceased, and the Gothlin Index had fallen to 6. There was no bleeding during the second 3 weeks, and none had been reported after 6 months.

The second case concerned a 20-year-old white female who had had massive pulmonary hemorrhages over a period of 3 years. These began 1 week after she had suffered a bump on the sternum in an automobile accident. There had been 6 recurrences, in which the bleeding was profuse. Medical and laboratory findings were negative. The Gothlin Index was increased, 10 petechiae appearing after the first stage of the test. She was placed on rutin, 20 mg. t.i.d. The bleeding ceased, and had not returned 8 weeks after treatment was begun. The Gothlin Index was then normal. The patient stated that she felt better than she had felt in 3 years.

Grelle (149) found that rutin, 10 mg. per diem given with ascorbic acid and vitamin K reduced the mortality from hemorrhagic disease of the newborn to 4.1 percent. Without rutin the mortality is 13.3 percent.

GLAUCOMA

In a series of papers, Stocker (126, 127, 128) has reported his studies on the use of rutin in glaucoma. The usual miotics, pilocarpine, physostigmine, and prostigmine, although they lower pressure in the anterior chamber by producing miosis, increase vascular permeability in the anterior chamber, and this effect tending to raise pressure, opposes the desired therapeutic action. In a series of patients with glaucoma, when rutin was given along with the miotic the pressure-lowering effect of the miotic was usually enhanced.

TOXEMIA OF PREGNANCY

Thirteen pregnant women with abnormal capillary fragility were treated with rutin by Dieckmann, Akbasli and Aragon (38); the starting dose was 20 mg. t.i.d. Capillary fragility was determined at 3-week intervals. If there was no improvement at the end of the first 3 weeks, the dose was doubled. When the index returned to normal, the patient was kept on a dose of 20 mg. t.i.d. In 12 of the 13 cases, rutin was effective in reducing the increased capillary fragility. In pregnant hypertensive patients, 60 mg. of rutin per diem was insufficient; in almost every case the dose had to be increased to 120 mg. per diem. No toxic symptoms were observed. The use of rutin in toxemia of pregnancy has also been reported by Shute (120).

HEMATURIA

Treatment of hematuria with rutin was described by Foucar (48) in a 44-year-old woman who had bilateral congenital polycystic kidneys with gross hematuria, marked impairment of kidney function, hypertension 210/150, and mild secondary anemia. As nothing could be done to improve the underlying lesion, an attempt was made to relieve the patient symptomatically. She was given 20 mg. of rutin and 50 mg. of ascorbic acid t.i.d. The bleeding stopped "promptly" and had not recurred in 6 months. The patient felt better and was working steadily. She was also relieved emotionally because of the suppression of the visible sign of her disability.

HYDROCELE

Foucar (48) also reports the successful use of rutin in a case of hydrocele of the right testicle in a 5-year-old boy.

EDEMA

A case of bilateral edema of the legs of 5 years' standing treated with rutin was reported by Foucar (47). Rutin, 20 mg., and ascorbic acid, 50 mg. were given t.i.d. In 12 days, there was less swelling, and after 5 weeks all edema had disappeared from the calves and ankles and there was slight swelling only on the dorsum of the feet.

RETINOPATHY

Medical experiences with the use of rutin in retinopathies have been summarized by Griffith (56). Shanno, Griffith and LaMotte (119) reported the results of rutin therapy in 73 patients with retinal hemorrhage associated with increased capillary fragility or permeability; their observations averaged 8 months (1 to 48). In 34 cases the capillary fragility returned to normal; in 11 it remained increased. The permeability became normal in 25 and remained increased in 8. There was no further hemorrhage in 61 of the patients. Twelve suffered another hemorrhage 1 to 7 months after treatment was begun. In these 12 cases, the capillary fault had not been corrected. Six of the patients were diabetic. There was a further hemorrhage in 3 cases.

C. W. Rutherford (115) states: "It is justifiable to give rutin as a prophylactic for progressive capillary weakness in cases of hemorrhage of the retina, even though weakness cannot be demonstrated by the special tests at the moment... Recurrences of retinal hemorrhages during or after administration of rutin do not condemn the drug nor warrant its discontinuance. Such occurrences suggest that the dosage be increased, or the presence of factors upon which rutin has no effect. Occurrence of subconjunctival hemorrhage while under treatment with rutin usually means a rise of both systolic and diastolic blood pressure and is not of itself alone an indication to discontinue the drug."

Donegan and Thomas (39) reported studies of 81 patients, 25 of whom had a retinopathy associated with increased capillary fragility.

Beardwood, Roberts and Trueman (9) studied 321 cases of diabetic retinopathies; 46 percent of them showed capillary fragility, and 24 percent showed evidence of retinal change. They were treated with rutin, hesperidin, and a placebo alternately. In 20 of 80 cases, cphthalmological examination revealed definite improvement -- there was either cessation of further evidence of bleeding or absorption of exudates and hemorrhages already present.

Rodriguez and Root (111) administered rutin to 70 diabetics with retinitis and increased capillary fragility, the majority of whom already had advanced disease. The capillary fragility could be brought to normal, but no improvement in the visual field or retinal picture was seen "despite the fact that several patients were strongly convinced that rutin had improved their vision."

MacLean and Brambel (88) used rutin with benefit in recurrent retinal and vitreous hemorrhage and in combination with dicumarol in venous thrombosis and the absorption of retinal hemorrhages. Two cases of Eales's disease were treated with rutin with good results. In one case the "improvement was almost dramatic. There was no more vitreous hemorrhage and the existing opacity cleared and absorbed rapidly with vision back to 20/30 in three weeks time."

In this connection Matis (161) obtained favorable results using rutin to counteract the tendency of dicumarol in some cases to impair the capillary wall manifested by bleeding. Determinations of the protein content of the capillary filtrate following use of dicumarol and again after treatment with rutin reveal that rutin is usually capable of counteracting the capillary impairment in six days.

A thorough consideration of the use of rutin in retinal hemorrhage has been published by Hollenhorst and Wagener (69), in which the literature is reviewed. They quote with apparent approval the statement of Rodriguez and Root: "Our studies show that capillary resistance is definitely low in practically all persons with diabetic retinitis. We suggest the desirability of further trial of this substance (rutin). More important, however, is the possibility that through further investigation of the pharmacology of rutin, we may ascertain the indications of its early use, in the hope of preventing the capillary damage related to the retinal and perhaps other degenerative complications in subjects with diabetes of long duration."

CAPILLARY PERMEABILITY

Capillary permeability is distinguished from capillary fragility by the fact that although both involve a fault of the capillary wall there is no fracture of the wall in permeability and no hemorrhage. Instead increased capillary permeability is characterized by an abnormal passage of fluid

through the intact wall, but cellular elements of the blood are retained. Capillary permeability may be measured by several methods. Two of these have been used to detect the effect of flavonoids: One, by observing the spread of a dyestuff injected intradermally and two, by observing the time required for the appearance of intravenously injected dyestuff in an irritated area (14).

The first method is used in a test developed by J. Q. Griffith, Jr., adapted from the technique of McMaster (95) in which a dyestuff, patent blue, is injected intradermally in the antecubital area and its spread noted at intervals for 15 minutes. If the dye spreads more than three-quarter inch in that time, it is considered to indicate an increased flow of lymph and, indirectly, increased permeability. Of a total of 279 patients with capillary fault reported by Griffith and Lindauer (Brochure, Chart 6, 1949), 81 had both increased capillary fragility and increased cutaneous lymphatic flow, and 31 had increased cutaneous lymphatic flow alone. The abnormal permeability is readily corrected by rutin, according to these authors.

Ambrose and DeEds (3), using albino rabbits, employed the second technique. Each animal received 2 ml. of a 1 percent tryphan blue, injected into the marginal ear vein. The depilated surface of the abdomen was irritated with chloroform approximately 5 minutes later, and the time of appearance of blue dye in the skin was noted. A rutin solution was then injected into the marginal ear vein, and 5 minutes later another area of the abdomen was irritated with chloroform. After doses of 100 or 200 mg./kg. of rutin there was a definite increase in the time required for the dye to make its appearance. The results were less marked after doses of 50 mg./kg. A few experiments indicated that rutin also delays the appearance of the dye in histamine wheals.

Küchmeister (154) successfully employed rutin to reduce capillary permeability in cases of essential hypertension, acute glomerular nephritis, peliosis rheumatica, malignant nephrosclerosis, inanition edema and central edema. When the rutin was discontinued relapses occurred.

Kuchinsky (156) perfused the hind legs of frogs and mice with Tyrode solution with and without the addition of rutin in concentration of 1-10,000 and 1-5000, respectively. In both species there was significantly less edema in the rutin animals than in the controls. He concludes that rutin hinders capillary permeability. In a later publication (157) with Dupont and Hennes these findings were extended.

BLEEDING GUMS

Strean (129) used a combination of 30 mg. of rutin and 50 mg. of ascorbic acid t.i.d. in 21 patients with bleeding gums either as a result of tooth brushing or following chewing of food. After 7 days, bleeding stopped in 12 patients; the number was increased to 15 after 2 weeks and then to 18 after 1 month. Three patients remained refractory.

MIGRAINE HEADACHE

Rutin has been used successfully in several cases of migraine, either alone or in combination with ascorbic acid and, where allergy was suspected, with some antihistaminic. No published reports of this application of rutin have come to our attention.

HEMOPHILIA

Rutin has been used to mitigate the severity of the symptoms in hemophilia under the direction of a number of physicians. Sixteen patients have been under observation for periods ranging from 2 to 5 years. The chief effects have been a reduction in joint hemorrhage, alleviation of pain, decrease in frequency and extent of hemorrhage after trauma, and fewer instances of necessity for transfusion. The parents of the hemophiliac children under treatment report great improvement in the patient's condition after treatment with rutin and insist upon continuing the treatment without lapse. In most of these cases, if not all, the patient is now receiving no other treatment. Many of the children whose schooling had previously been seriously interrupted have been able to resume attendance, with a minimum of absence due to sickness.

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ERRATA

Chemistry, Pharmacology and Clinical Applications of Rutin. AIC-291

Page 9, line 33. Cronkeit should be Cronkite.

Facing page 5, add Fig. 1; following figure (next page), add Fig. 2.

Literature Cited, p. 25-30:

5. Anderson should be Andersen.

7. Armentano, P. L., should be Armentano, L.

8. 324-340 should be 234-240.

11. Beiler, J. M., and Martin, G. J., should be Beiler, J. M., Brendel, R., Graff, M., and Martin, G. J.

16. Bustinza, L. F., and Lopez, L. A. should be Bustinza, F., and Lopez, A. C. 548-559 should be 549-559.

22. Groves should be Grover.

23. Add: See footnote 5, p. 17, loc. cit.

34. Standford should be Stanford.

51. Sept. 4 should be Sec. 4.

52. 1-6 should be 1-8.

53. 104 should be 105.

55. Add: 434-438.

75. Add: Sci. Ed. before 37.

76. (June 7, 1948) should be (Nov. 5, 1948).

89. Add: See footnote 5, p, 17, loc. cit.

95. 347 should be 347-372.

112. Sheppard should be Shepperd.

121. p. 58 should be 58 pp, after Monograph, add No. I.

122. p. 54 should be 54 pp, after Monograph, add No. I.

131. Pharm. Zentralh. 13 should be Pharm. Zentralbl. 13 (II)

137. Zfass, H. A. should be Zfass, H. S.

144. Cronkeit should be Cronkite.

145. Cronkeit should be Cronkite. Report no. is no. 19.

150. Add: UCLA 86. after Report. p. 18 should be 18 pp.

153. Ref. should read: Koenigsberg, M., West Va. Med. J. 44:235-238 (1948).

157. 142 should be 143.

164. 2423-2427 should be 3423-3427.